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Simulated space radiation: Investigating ionizing radiation effects on the stability of amlodipine besylate API and tablets

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ABSTRACT

Efficacious pharmaceuticals with the adequate shelf life are essential for the well-being of the space explorers and successful completion of a space mission. Space is brimming with different types of radiations, which penetrate inside the spacecraft despite the shielding material. Such radiations can alter the stability of the pharmaceuticals during long duration space missions. The literature reporting the space radiation effects on the pharmaceuticals is scarce in a public domain. Ground-based simulation studies can be useful to predict the influence of the space radiations on the stability of the pharmaceuticals. Based upon these facts, the main objective of the present preliminary work was to investigate the effect of different types of ionizing radiations on the stability of amlodipine besylate API and tablets. Amlodipine besylate samples were irradiated by protons, neutrons (thermal and fast), gamma and heavy ion (^{56}Fe) radiations with their different doses. The samples were also irradiated with UV–visible radiation to compare the effect of selected ionizing radiations with photodegradation. The physical stability was examined through organoleptic evaluation and the chemical stability was evaluated by FTIR and HPLC. The results of the organoleptic evaluation showed colour changes from colourless to yellow in proton irradiated solid API and gamma irradiated API aqueous solution. The FTIR spectrum of proton irradiated API showed one additional absorption band at 1728 cm^{-1} due to degradation products. HPLC analysis revealed that amlodipine degraded up to 10% and 21% after the highest doses of proton and gamma irradiation, respectively. No physical or chemical changes were observed after neutron and ^{56}Fe irradiation. The structures of major radiolytic products were elucidated using LC-MS/MS. Two new impurities were found in the API aqueous solution as a result of gamma irradiation. The drug degradation pathways were postulated by proposing the plausible mechanism of formation.

1. Introduction

Stable pharmaceuticals play a crucial role in the health-care system of human beings, equally on the Earth and in the space. Instability may affect the quality of the product – its safety, potency and efficacy (Carstensen, 1974). It is therefore essential to maintain the stability throughout their shelf-life. Space medical records outline the cases of reduced efficacy of pharmaceuticals during several space missions (Wotring, 2015). The possible reasons are altered human physiology i.e. changes in absorption, distribution, metabolism and excretion (ADME) of drugs and physicochemical instability of pharmaceutical dosage forms in presence of space environment (Du et al., 2011; Tietze and Putcha, 1994; Marshburn, 2008).

Du et al. evaluated the stability of the pharmaceuticals stowed inside the International Space Station (ISS) versus their ground control samples. They revealed that the samples retrieved from ISS were less stable than their ground control samples. Among total 35 samples, only 9 samples from ISS whereas 17 samples from ground control samples were found in accordance with USP acceptance criteria of stability. The rate of drug degradation was also higher in space, especially for light-sensitive drugs such as promethazine, dextroamphetamine, ciprofloxacin (Du et al., 2011). Another article reported that four months storage of different brands of vitamin B complex tablets in the ISS resulted in significant degradation of vitamin B_3 in one of the brands (Chuong et al., 2011). Space environment was a possible reason.

Generally, the stability of pharmaceutical dosage forms is evaluated by accelerated and real-time stability studies as per various regulatory guidelines such as ICH, WHO, FDA etc. (*Annex 2, Stability testing of active pharmaceutical ingredients and finished pharmaceutical products,* 2009, "Food and Drug Administration. Guidance for Industry Q1A(R2)

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Stability Testing of New Drug Substances and Products,", 2003, "Q1A(R2) ' Stability Testing of New Drug Substances and Products', International Conference on Harmonisation, Geneva,", 2003). These guidelines are developed according to terrestrial environmental conditions. But, the acceptance criteria of these guidelines may not assure the stability of medicines during long-duration space missions as the spaceflight environment is different than the Earth. The unique environmental factors of the spaceflight include varying temperature and humidity, microgravity, vibration and excessive radiation (Thirsk et al., 2009). Among these factors, microgravity seems to be a less problematic factor for instability of medicines. The temperature and humidity conditions of the spaceflight are always controlled and regulated at ambient conditions. But, the cumulative dose of space radiation inside the spaceflight is high as well as different than on the Earth (Mehta and Bhayani, 2017). Therefore, the space radiation is believed to be the biggest safety concern during space travel - whether for astronauts or any other material inside the spacecraft including pharmaceuticals. Research work regarding the effect of cosmic radiations encountered during space missions on the stability of drugs is very limited.

The invisible shields of the Earth, namely magnetosphere and atmosphere, protect us from highly ionizing space radiation. Mainly UV–visible radiation affects the stability of medicines among Earth's radiation environment (Bagshaw, 2008; Kamide, 2000). The space radiation includes Galactic Cosmic Rays (GCR), Solar Cosmic Rays (SCR) and particles trapped by the Earth's magnetic field (Reitz et al., 1995; Stassinopoulos and Raymond, 1988) (Composition in supplementary material Fig. S1). These space radiations penetrate inside the spacecraft despite shielding material during space travel. Moreover, the interactions of these radiations with spacecraft shielding material produce secondary radiation (Medina, 2015). Therefore, the possible effects of the space radiation i.e. drug degradation, chemical structures and toxicity of degradants, drug degradation pathways etc. should be known to assure the quality of medicines during long-duration space missions.

It is challenging to perform a systematic real-time stability study for pharmaceuticals in a space radiation environment. The major constraint in this is the storage of a large number of pharmaceuticals inside the spacecraft owing to limited space and restricted mass/volume that can be flown on spacecraft. It is also difficult to reimburse stored pharmaceuticals from the spacecraft at different time intervals. In these circumstances, the ground-based analogue study can be useful to estimate the possible effects of radiation on pharmaceuticals. The obtained results from the ground analogue study can be compared to available data of drug degradation during spaceflight in order to develop accelerated stability protocol for space radiation environment. It will enable to predict the shelf life of the formulations. But, before ground analogue study it would be worthwhile to perform experiments similar to forced degradation study to assess the susceptibility of pharmaceuticals to highly ionizing radiation present in spaceflight. Generally, forced degradation study is carried out to understand the chemical behaviour of the drug, to identify possible degradants and drug degradation pathways. Similar studies by exposing the drugs to different ionizing radiations at different doses would provide knowledge regarding the possible interaction of ionizing radiations with drugs and to predict degradation behaviour of pharmaceuticals during spaceflights.

Based upon these facts, the research objective of this preliminary study was intended to evaluate the effect of different types of highly ionizing radiations including protons, neutrons (thermal and fast), gamma and iron (^{56}Fe) on the stability of selected pharmaceuticals. Also, to investigate whether the ionizing radiations have the same degradation effect on the pharmaceuticals like UV–visible radiation or they lead to different degradation pathways. These radiations were selected for the study either due to their proportional dominance in space radiation or due to their damaging capabilities. The protons account for more than ~ 85% of deep space radiation (Pecaut et al., 2002). The neutrons are difficult to shield and they penetrate up to longer distances in the spacecraft (Kuhne et al., 2009). Interactions of space radiations with spacecraft shielding material produce neutron and gamma radiations as secondary radiations (Aharonian, 2004; Haskins et al., 1992). Although the fraction of heavy ions in space radiation is very less (~1%), they are considered harmful because of their very high energy. Iron (Fe) is the most important among all heavier ions owing to its abundance and high Linear Energy Transfer (LET). Compared to protons, the abundance of Fe is one thousandth, but, they have equal dose contribution due to the Z^2 dependence (Elmore et al., 2011; Jones and Karouia, 2008).

Amlodipine besylate, classified as a calcium channel blocker, was selected as a model drug for the present work. It is prone to photodegradation. (Jakimska et al., 2014). Photolabile drug was selected to compare radiation degradation profile with photodegradation to know the presence of radiation generated unique impurities. Amlodipine besylate API and its tablets were exposed to a different dose of the proton, neutron, gamma, ^{56}Fe radiations and ICH recommended UV–visible radiation, simultaneously. The physical changes were examined by organoleptic evaluation. The chemical changes were analysed using Fourier-Transform Infrared spectroscopy (FTIR) and High-Performance Liquid Chromatographic (HPLC) method and major degradation products were identified by using mass spectrometry and their possible degradation pathways were postulated.

2. Experimental

2.1. Material

The reference standard of amlodipine besylate API was procured as a gift sample from Zydus research centre, Ahmedabad. Marketed tablets of amlodipine besylate, Amlip 5 (Cipla Ltd., Baddi, India) were purchased from local pharmacies. HPLC grade methanol, acetonitrile, glacial acetic acid, ammonium acetate buffer and Milli-Q water were purchased from Merck, Mumbai.

2.2. Methods

API and tablets of amlodipine besylate covered in aluminium foil and stored at 20–25 °C /60–65% RH served as control samples.

2.2.1. Photodegradation study

The photostability studies were carried out in a photostability chamber (Thermolab Scientific Equipment, Mumbai, India) as per light sources recommended in ICH Q1B guideline. The photostability chamber was equipped with two black light UV lamps, which had spectral distribution in UV-A range (320–400 nm) and four white fluorescent lamps, which produced an output similar to that specified in International Organization for Standardization (ISO) 10,977(1993). Amlodipine besylate API in solid state was kept in an open glass petridish and API aqueous solution (1000 µg/mL prepared in water) was filled in quartz vials as the ordinary glass vial is not transparent for UV light. Each sample was placed in the photostability chamber and irradiated by 200 W*h/m^2 UV radiation as well as 1.2 million lux hours visible radiation simultaneously.

2.2.2. Radiation exposure

Pure API and intact tablets of amlodipine besylate were irradiated in the solid state by all selected ionizing radiations i.e. protons, neutrons, gamma and ^{56}Fe radiations (Fig. 1). For gamma irradiation, the aqueous solutions of API (1000 µg/mL) were also irradiated along with solid API and tablets. But, in the other radiation sources, irradiation of the liquid sample was not feasible.

Proton irradiation was carried out at Folded Tandem Ion Accelerator (FOTIA), Bhabha Atomic Research Centre (BARC), Mumbai with doses of 50, 200, 500 Gy and 5 kGy using ~5 MeV protons at room temperature (RT) under high vacuum (10^{-6} Torr) conditions. One set

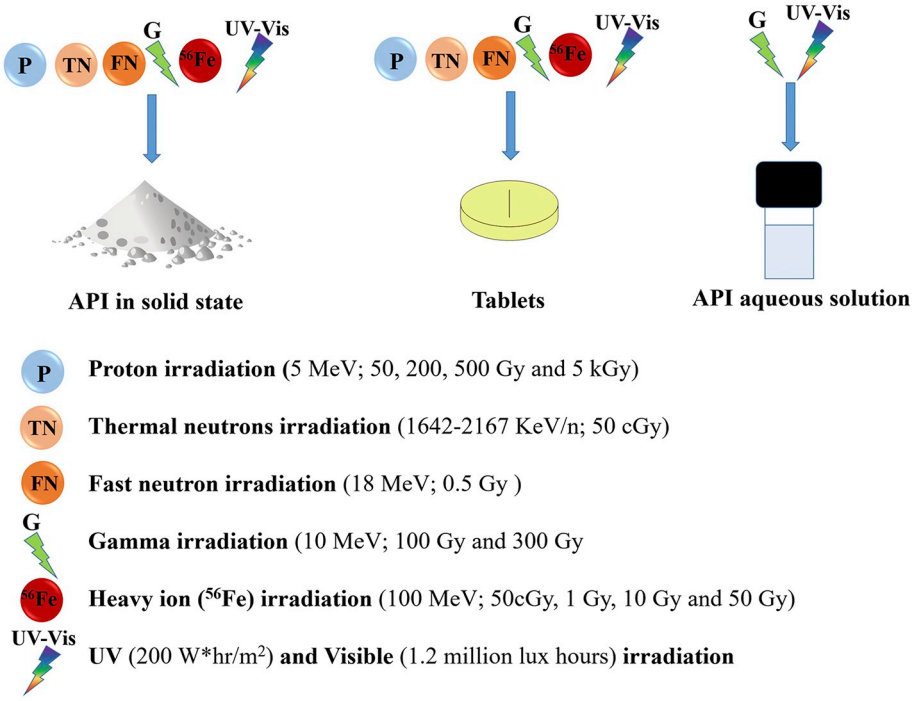

P
TN
FN
G
56Fe
UV-Vis
API in solid state
Tablets
API aqueous solution
Proton irradiation (5 MeV; 50, 200, 500 Gy and 5 kGy)
Thermal neutrons irradiation (1642-2167 KeV/n; 50 cGy)
Fast neutron irradiation (18 MeV; 0.5 Gy)
Gamma irradiation (10 MeV; 100 Gy and 300 Gy
Heavy ion (56Fe) irradiation (100 MeV; 50cGy, 1 Gy, 10 Gy and 50 Gy)
UV (200 W*hr/m2) and Visible (1.2 million lux hours) irradiation

Fig. 1. Amlodipine besylate samples irradiated by ionizing radiations.

of samples was irradiated by thermal neutrons (1642-2167 KeV/n, 50 cGy radiation dose, at RT) at Critical Facility, BARC. Another set of samples was irradiated by fast neutrons (18 MeV, 0.5 Gy radiation dose, at RT) at Pelletron Accelerator, Tata Institute of Fundamental Research (TIFR). ^{56}Fe irradiation (^{56}Fe, 100 MeV with 50 cGy, 1 Gy, 10 Gy and 50 Gy doses at RT) was carried out at Inter University Accelerator Centre (IUAC), Delhi. The gamma radiation exposure (10 MeV/n, with doses of 100 Gy and 300 Gy at RT) was performed at Electron Beam Centre (EBC) at Kharghar, Mumbai. All the irradiated samples were stored in controlled environmental conditions of 20–25 °C temperature and 60–65% RH until the time of analysis.

2.2.3. Organoleptic analysis

The samples were examined for their physical appearance, colour, odour, solubility as well as clarity of solution before and after irradiation.

2.2.4. Fourier-transform infrared spectroscopy

FTIR spectra of controlled and irradiated API were recorded by FT/IR-1800 spectrometer (JASCO Corporation, Japan) equipped with Spectra manager software using diffuse reflection method. The spectra were recorded using KBr with a scanning range from 4000 to 400 cm^{-1} and a resolution of 4 cm^{-1}.

2.2.5. High-performance liquid chromatographic method

The stability of samples in terms of percentage degradation was analysed using 1260 Infinity Quaternary HPLC system (Agilent Technologies, United States), which was having auto-sampler of 100 µL capacity loop and equipped with OpenLAB CDS EZChrom software (V A.01.03). The chromatographic conditions for separation of the drug and degradation products were adopted from Tiwari et al. (2015). The phenomenex Gemini C_{18}, (250 × 4.6) mm; 5 µm column was used at 40 °C for the separation of the impurities and the drug. The mobile phase consisted ammonium acetate buffer (pH 5; 10 mM) and methanol in gradient mode [$Time_{(min)}$/%methanol: T_0/10; T_8/50; T_{13}/60; T_{25}/75; T_{27}/10; T_{30}/10] at a flow rate of 1.0 mL/min. The injection volume was 20 µL and the detection wavelength was kept at 240 nm. Controlled and irradiated samples of amlodipine besylate were accurately weighed, dissolved in water and finally diluted by mobile phase to produce a concentration of 1000 µg/mL for impurity profiling. Each sample was analysed in duplicate using HPLC system for content analysis.

2.2.6. Mass spectrometry

The radiolytic degradation products were identified by molecular weights using Xevo® G2 Q-TOF mass spectrometer (Waters Corporation, United States) coupled to Acquity UPLC System. Electrospray ionization (ESI) interface was used in a positive mode. Instrument control and data acquisition were performed using MassLynx software (V 4.1). The ion source parameters were optimized as follows: 3.00 kV capillary voltage, 40 V cone voltage, 350 °C desolvation temperature, 150 °C source temperature, 800 L/h desolvation gas (N_2) flow rate and 50 L/h cone gas flow rate. The chromatographic conditions were kept same as mentioned in section “High-performance liquid chromatographic method”.

3. Results and discussion

API and tablets of amlodipine besylate were exposed to ionizing radiations namely protons, neutrons, gamma and ^{56}Fe with different doses to evaluate their impact on the physicochemical stability of the drug. Moreover, amlodipine besylate API samples in solid and aqueous solution state were irradiated by UV–visible radiation as per ICH Q1B guideline. Controlled and irradiated samples were analysed simultaneously. The physical parameters such as colour, odour, appearance, solubility etc. were examined by organoleptic evaluation. The chemical changes were evaluated by FTIR and HPLC. The radiation degradation profile of amlodipine besylate was compared to the photodegradation profile to check the presence of radiation generated unique impurity. The impurities present above the 0.1% level were reported and the major degradation products were identified using LC-MS/MS.

3.1. Organoleptic evaluation

Initially, organoleptic evaluation of the all irradiated samples was carried out. No changes were found in the odour, the solubility of amlodipine besylate in water and methanol as well as clarity of solution

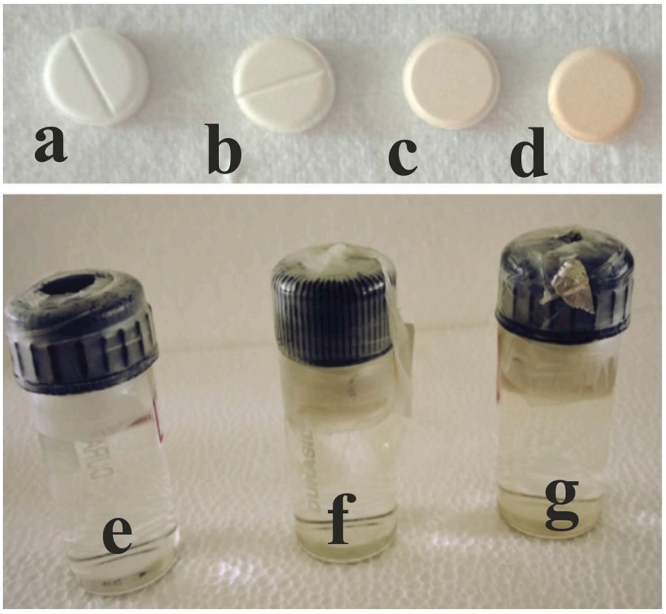


Fig. 2. Physical changes observed in tablets (a) controlled and after proton irradiation (b) 50 Gy (c) 200 Gy (d) 5 kGy; API aqueous solution (e) controlled and irradiated by gamma radiation (f) 100 Gy (g) 300 Gy.

in the samples irradiated by all selected ionizing radiations. However, colour changes were observed in the amlodipine besylate API samples after irradiation by proton, gamma and UV–visible radiations. Initially, the colour of solid API was white, which changed to yellowish brown after exposure of UV–visible radiation. The API aqueous solution also turned to yellow in colour after UV–visible irradiation. Proton irradiated API samples with 50, 200, 500 Gy and 5 kGy doses changed to pale yellow, yellow, dark yellow and yellowish brown, respectively. Upon exposure of gamma radiation, solid API samples did not show any colour change at selected doses, but, the aqueous solution of API became more intense in colour compared to the controlled sample (Fig. 2). Neutron and ^{56}Fe irradiated API samples did not indicate any colour changes.

For the proton irradiated tablet formulations, the physical changes accelerated at the exposed surface. The colour of irradiated tablets changed to pale cream from white, which darkened with an increase in radiation dose (Fig. 2). Tablets exposed to gamma, neutrons and ^{56}Fe radiation showed no discolouration.

The organoleptic evaluation indicated that amlodipine besylate was sensitive to proton and gamma irradiations as well as UV–visible radiation. The observed colour changes might be a consequence of the formation of the coloured product, radiolytic products that absorbs visible radiation or by the formation of “colour-center”. The displacement of an electron from its occupied position generates “hole colour-center” and the presence of extra electron trapped in a solid matrix leads a formation of “electron colour-center”. These “colour-centers” absorb visible radiation causing colour changes (West, 2014).

3.2. FT-IR analysis

At the next stage of the study, the samples were subjected to FT-IR analysis to determine the structural change of amlodipine besylate before and after irradiation with UV–visible, proton, neutron, gamma and ^{56}Fe radiations. As shown in Fig. 3, the recorded FT-IR spectra of controlled and irradiated samples were in full agreement with each other and there were no changes in the characteristic absorption bands except in proton irradiated samples with the highest dose (5 kGy). The characteristic absorption bands of amlodipine besylate were observed at 3293 cm^{-1} (N—H stretches of amine, dihydropyridines and ammonium group); 1697 and 1673 cm^{-1} (C=O stretches of ester carbonyl); 1093 cm^{-1} (aromatic C—Cl stretch); 1033 and 1018 cm^{-1} (ether C-O-C symmetric stretch); 754, 728 and 615 cm^{-1} (aromatic C—H bending) in all samples (Ananchenko et al., 2012). One additional weak band was observed in proton irradiated API samples at 1728.87 cm^{-1}, might be due to the presence morpholine ring containing impurities (Rapolu et al., 2014; Reddy et al., 2010). The results obtained from FTIR study indicated that radiation exposure either did not affect the amlodipine besylate structure or radiolytic products may have a similar structure with parent structure.

3.3. Radiolytic products analysis by HPLC

All the irradiated and controlled samples were analysed by HPLC to examine the influence of the ionizing radiation on the chemical stability of the API and tablets of amlodipine besylate. Moreover, the chromatographic profiles for photodegraded amlodipine besylate API in both solid and solution state were compared to amlodipine besylate irradiated by other selected ionizing radiations in order to identify ionizing radiation generated unique impurity.

As illustrated in Fig. 4, the chromatographic peak of amlodipine was observed at Retention time (R_t) 19 min in all samples. The controlled API samples showed all impurities below the 0.1% level [two impurities at Relative Retention Time (RRT) 0.88 (IMP6) and 0.91 (IMP7), respectively] while controlled tablet samples displayed impurity at RRT 0.91 (IMP7) up to 0.1%. It indicated that IMP7 was common in both API and tablets.

During photolytic studies, drug degraded to a lesser extent (up to 1.09%) in the solid-state conditions. The chromatogram for photodegraded API in the solid-state showed an additional major peak at RRT 0.82 (1.01%, IMP5). Whereas, in the aqueous solution amlodipine degraded up to 7.86% under photolytic conditions. The chromatogram of the photodegraded aqueous solution of amlodipine besylate displayed three major degradation products at RRT 0.65 (0.22%, IMP2), 0.75 (2.66%, IMP4), and 0.82 (4.18%, IMP5) among total four impurities.

The samples were irradiated by proton radiation only in the solid state. Dose-dependent percentage degradation of amlodipine was observed in API and tablets irradiated by protons, wherein the percentage degradation increased as the dose of proton radiation increased. (Fig. 4a) The chromatogram for proton irradiated API with 5 kGy dose showed a total of twelve impurities (> 0.1%) that accounted total 10.53%. Four major impurities were noted at RRT 0.82 (IMP5), 0.88 (IMP6), 1.32 (IMP13) and 1.46 (IMP15). The amount of these major impurities was found to be 4.47%, 0.50%, 3.64% and 0.82%, respectively. The chromatogram for proton irradiated tablets with 5 kGy dose displayed 0.97% drug degradation with a total of five impurities (> 0.1%) (Fig. 4b). Three major impurities were detected at RRT 0.40 (0.14%, IMP16), 0.82 (0.30%, IMP5) and 1.32 (0.28%, IMP13). The RRT with the percentage of each degradation product is summarized in Table 1. The impurities IMP5 and IPM13 were common in both API and tablet samples irradiated by protons. However, IMP16 was present only in proton irradiated tablets. It was neither seen in the proton irradiated API nor in any photodegraded samples. It indicated that IMP16 might have generated from amlodipine besylate in presence of excipients or it might have generated from excipient itself on proton irradiation. Among all proton generated degradation products, only IMP5 was found to be present in photodegraded amlodipine besylate in a solid and aqueous solution state. But, other degradation products formed by proton irradiation were not observed in photodegraded samples (Fig. 4d).

The chromatograms of amlodipine besylate samples irradiated by gamma radiation displayed significant degradation in the aqueous solution state. In the aqueous solution, amlodipine degraded up to 21.23% upon 300 Gy gamma irradiation, wherein total eleven out of thirteen radiolytic products were noted as major degradation products in the HPLC chromatogram (Fig. 4c; Table 1). Among these degradation

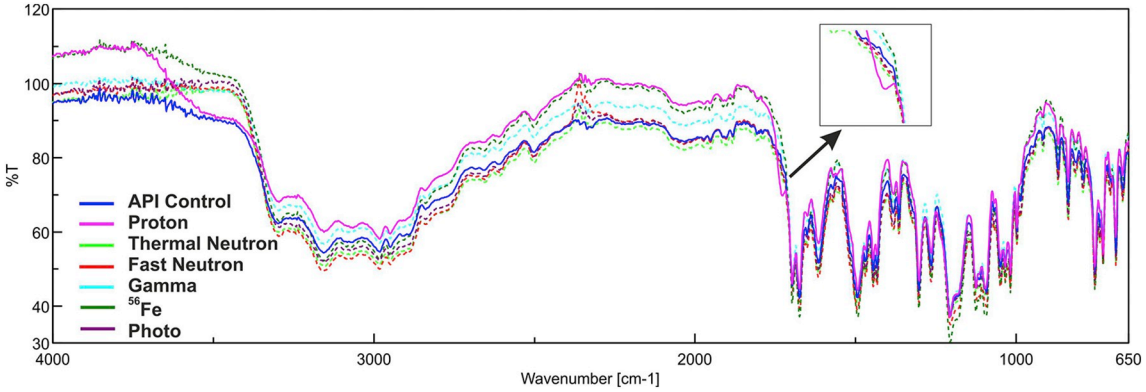


Fig. 3. FTIR spectra of controlled and irradiated amlodipine besylate API.

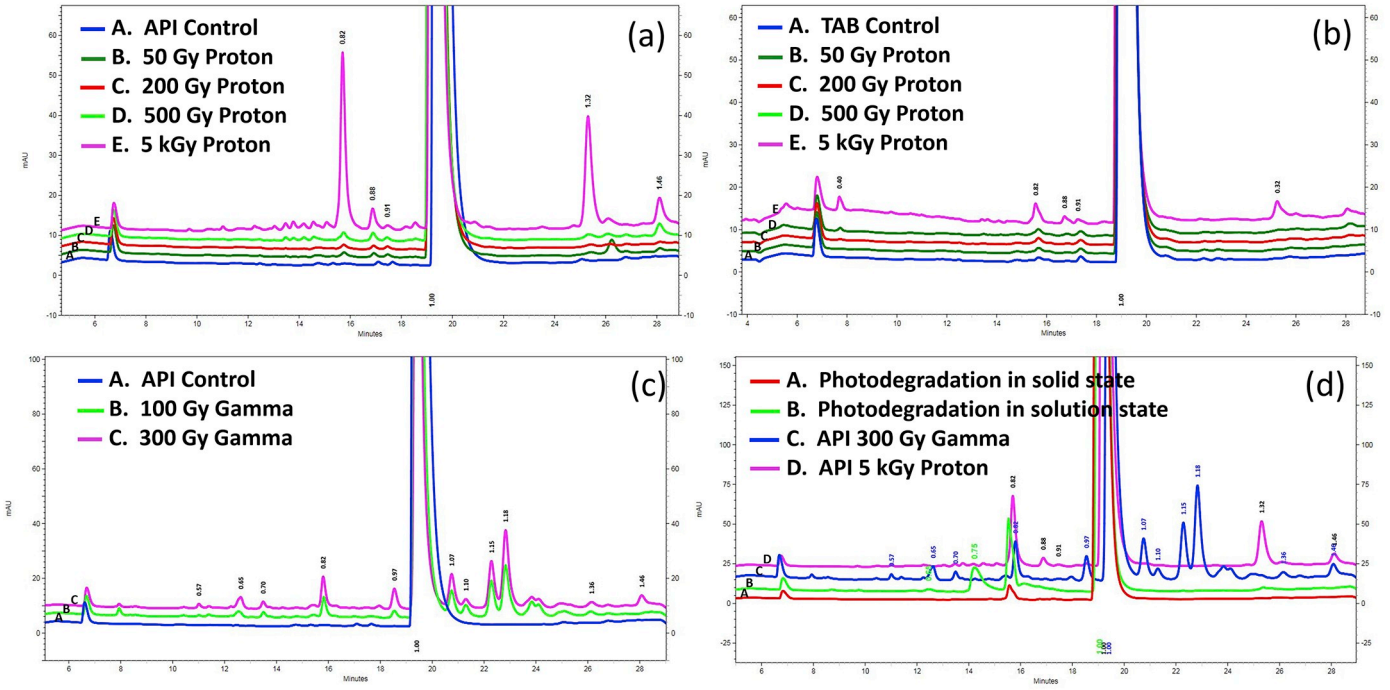


Fig. 4. HPLC chromatograms of amlodipine besylate (a) API irradiated by proton radiation (b) tablets irradiated by proton radiation (c) API aqueous solution irradiated by gamma radiation (d) Overlay of API irradiated by proton, gamma and UV–visible radiations.

products, IMP2 and IMP5 were the same as observed in photodegraded amlodipine besylate aqueous solution. But, other gamma induced degradation products were different from photodegradation products (Fig. 4d). It suggested that the major degradation products generated due to gamma irradiation were new and were not observed on photodegradation of amlodipine besylate.

IMP5 was found as a one of the major degradants in all irradiated conditions i.e. proton, gamma and UV–visible. The comparison of chromatographic profiles of proton and gamma irradiated samples showed that the degradation products such as IMP3, IMP5, IMP7, IMP8, IMP14 and IMP15 were common in both. But, the peak area ratios of these degradation products were different in their chromatographic profiles. This indicated that the drug degradation behaviour under each irradiation condition was different. These degradants might have generated by different degradation pathways or different mechanisms have followed to generate these degradation products from amlodipine besylate.

The gamma irradiated API in the solid state and tablets exhibited no degradation in their chromatograms. This indicated the amlodipine besylate stability towards gamma radiation in the solid state. The HPLC chromatograms of amlodipine besylate API and tablets irradiated by thermal neutrons, fast neutrons and ^{56}Fe radiation exhibited no additional peak compared to control samples, as well as the peak areas of amlodipine remained unchanged. These results indicated that amlodipine besylate was chemically stable to these radiations at selected doses. (Chromatograms are shown in the supplementary material Fig. S2).

It is noteworthy that the photodegraded and gamma irradiated solid API was found to be stable compared to the irradiated API aqueous solution. But, the proton irradiated API degraded significantly despite the solid state. This suggested that proton radiation prominently affects the stability of amlodipine besylate. Moreover, total %degradation in tablets after proton irradiation was less or negligible than the API. It might attribute to the limited radiation penetration inside the tablets, where the drug present only in the top layers degraded by the proton radiation, but, the inner core remained intact and the drug degradation by proton radiation was prevented.

Table 1
Degradation summary of amlodipine besylate irradiated by proton, gamma or UV–visible radiation.

Radiation	Sample	% degradation	Numbers of degradation products above 0.1%	degradation products [RRT; %degradation]	
				Major	Minor
Proton	API (Solid)	10.53	12	IMP5 [0.82; 4.47%], IMP6 [0.88; 0.50%], IMP13 [1.32; 3.64%], IMP15 [1.46; 0.82%]	[0.70; 0.10%], [0.72; 0.10%], [0.74; 0.10%], [0.76; 0.15%], [0.79; 0.10%], [0.91; 0.10%], [0.97; 0.12%], [1.36; 0.16%]
	Tablet	0.97%	5	IMP16 [0.40; 0.14%], IMP5 [0.82; 0.30%], IMP13 [1.32; 0.28%]	[0.88; 0.11%], [1.46; 0.10%]
Gamma	API (Aqueous Solution)	21.23%	13	IMP1 [0.57; 0.20%], IMP2 [0.65; 0.81%], IMP3 [0.70; 0.31%], IMP5 [0.82; 1.84%], IMP8 [0.97; 1.54%], IMP9 [1.07; 3.08%], IMP10 [1.10; 0.72%], IMP11 [1.15; 3.97%], IMP12 [1.18; 7.12%], IMP14 [1.36; 0.43%], IMP15 [1.46; 0.77%]	[0.41; 0.12%], [0.91; 0.19%]
Photo	API (Solid)	1.09	1	IMP5 [0.82; 1.01%]	–
	API (Aqueous Solution)	7.86	4	IMP2 [0.65; 0.22%], IMP4 [0.75; 2.66%], IMP5 [0.82; 4.18%],	[0.85; 0.2%]

3.4. LC-MS/MS analysis

The degradation products were not isolated in pure form due to their very small quantity, hence, the degradation products were tried to characterize on the basis of mass fragmentation pattern through LC-MS/MS studies. The mass spectral analysis was carried out in a positive mode using electrospray ionization. Among total sixteen major radiolytic products generated by radiation exposure, total ten were identified using LC-MS/MS. The isotopic pattern [M + 2 + H*] observed in the mass spectra confirmed the presence of an intact chlorine atom (Cl) in the degradants. The summary of the identified impurities is given in Table 2.

Initially, the mass fragmentation behaviour of the parent drug was investigated for a better understanding of degradation product fragmentation. The mass spectrum of amlodipine besylate showed a protonated molecule peak at mass-to-charge ratio (*m/z*) 408.80 in a positive mode corresponding to its molecular mass of 408 Da. The MS/MS spectrum of this precursor ion (*m/z* 408.80) showed product ions at *m/z* 392.07, 345.92, 294.04, 238.02, 208.10. The fragment ion at *m/z* 392.07 was generated by the loss of ammonia from amlodipine. The ion at *m/z* 345.92 was formed by the subsequent loss of ethanol. The product ions at *m/z* 294.04 and 238.02 were generated through molecular rearrangements and atypical ring cleavage of the dihydropyridine moiety (Gibbons et al., 2006; Handa et al., 2017; Yasuda et al., 1996). The peak at *m/z* 142.12 was generated by the loss of methanol, hydrochloric acid and carbon monoxide from *m/z* 238.02. Formation of the fragment at 208.10 resulted due to the loss of ethanamine, ethanol and chlorobenzene from *m/z* 409. The fragmentation scheme and fragmentation pathways are given in the supplementary material Fig. S3 and S4.

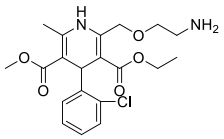
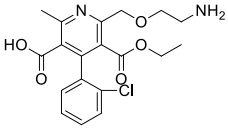
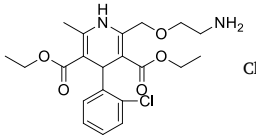
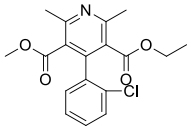
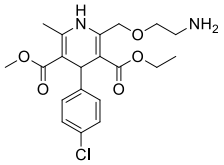
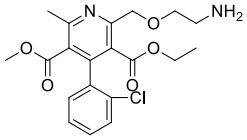
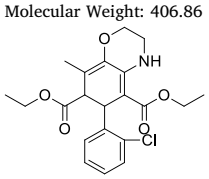
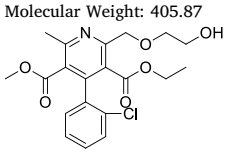
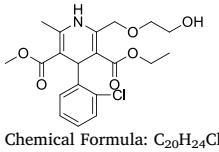
In the LC-MS spectrum of IMP1, protonated molecule peak was detected at *m/z* 393.29. It indicated the loss of the methyl group from the parent ion (amlodipine). A similar impurity has been reported as an alkaline and acidic hydrolytic degradation product in the literature (Bodapati et al., 2015; Suchanova et al., 2006). IMP2 showed *m/z* 423.31, which was 14 mass units higher compared to the precursor ion of amlodipine. It is listed as impurity E in the European Pharmacopoeia (Gibbons et al., 2006; Sudhakar et al., 2006). IMP3 was noted at *m/z* 349.24 in the LC-MS spectrum, indicating the loss of ethanolamine from amlodipine. It is one of the known photodegradation products (Jakimska et al., 2014; Zhu et al., 2015).

The $[M + H]^+$ peak of radiolytic product IMP4 was observed at *m/z* 409.32, which was similar to amlodipine. It is an isomer of amlodipine, which has been reported in the available literature as a process related impurity (Sudhakar et al., 2006). In the LC-MS spectrum of IMP5, $[M + H]^+$ peak was noted at *m/z* 407.31. IMP5 corresponded to the pyridine analogue of amlodipine. It is listed as an impurity D in the European Pharmacopoeia and as the impurity A in the United States Pharmacopeia. It is also reported as the acid, oxidative or photodegradation product in the various literature (Bodapati et al., 2015; Jakimska et al., 2014; Tiwari et al., 2015; Zhu et al., 2015). IMP5 formed due to aromatization of dihydropyridine moiety by radical cation mechanism. IMP6 displayed $[M + H]^+$ peak at *m/z* 406.28. IMP6 was formed by molecular rearrangements and cyclization (Béni et al., 2015). The mass spectrum of IMP9 and IMP11 showed $[M + H]^+$ peak at *m/z* 408.30 and 410.31, respectively. In both the impurities, the amine group present in the side chain of amlodipine was substituted by the –OH group. IMP9 is a pyridine analogue of IMP11, which has a dihydropyridine moiety in its structure. To the best of our knowledge, these impurities are not reported in the literature.

Protonated molecule peak for IMP12 was detected at *m/z* 348.25. There are two possible structures for IMP12, one with a Cl atom and another without a Cl atom. But, no characteristic Cl isotope pattern was observed in its mass spectrum. Hence, IMP12 could be generated by the loss of aminoethyl group from side chain and substitution of the - OH group at the place of Cl atom. This impurity has been reported as a phototransformation product (Jakimska et al., 2014; Zhu et al., 2015). IMP13 was noted at *m/z* 360.26 in the LC-MS spectrum, indicating loss of ammonia, intramolecular rearrangement and loss of ethanol from the amlodipine (Reddy et al., 2010). Due to the poor ionization IMP7, IMP8, IMP10, IMP14, IMP15 and IMP16 were not identified by LC-MS analysis, which might require further assistance from other hyphenated techniques.

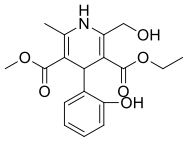
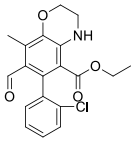
The degradation pathways of the radiolytic products are elucidated

Table 2
Proposed radiolytic products of amlodipine besylate.

Peak	RRT	Measured mass (*m/z*)	Proposed chemical structure	MS/MS fragmentation (*m/z*)	Observed in irradiated conditions
Amlodipine	1	409.3	 Chemical Formula: $C_{20}H_{25}ClN_2O_5$ Molecular Weight: 408.88	392.07 (-NH_3), 208.10 (-$OHC_2H_4NH_2$; C_2H_5OH; C_6H_5Cl) 294.04, 238.02, 142.12 (molecular rearrangements and atypical ring cleavage of the dihydropyridine moiety),	–
IMP1	0.57	393.29	 Chemical Formula: $C_{19}H_{21}ClN_2O_5$ Molecular Weight: 392.83	377.82 (-NH_3), 332.21 (-$OHC_2H_4NH_2$), 288 (-$OHC_2H_4NH_2$;-C_2H_2OH), 231.11 (-$OHC_3H_6NH_2$; –C_2H_2OH;-CO;-H_2O), 167.14 (-C_6H_5Cl; –$HCOOC_2H_3$; -C_2HNH_2)	Gamma
IMP2	0.65	423.31	 Chemical Formula: $C_{21}H_{27}ClN_2O_5$ Molecular Weight: 422.90	377.30 (-C_2H_5OH), 334.23 (-C_2H_5OH; -$C_2H_4NH_2$), 288.16 (-C_2H_5OH; -$C_2H_4NH_2$; –C_2H_5OH), 238.17 (-CH_3; -$C_8H_{13}NO_3$), 208.16 (-CH_3; $C_2H_5NH_2$; C_2H_5OH; C_6H_5Cl), 176.11 (-CH_3; $C_2H_5NH_2$; C_2H_5OH; C_6H_5Cl; –CH_3OH)	Gamma, UV-$Vis_{solution}$
IMP3	0.70	349.24	 Chemical Formula: $C_{18}H_{19}ClNO_4$ Molecular Weight: 348.80	260.13 (-CH_3COOH; –C_2H_5OH) 230.13 (-CH_3COOH; –C_2H_5OH; – CH_3OH)	Gamma
IMP4	0.75	409.32	 Chemical Formula: $C_{20}H_{25}ClN_2O_5$ Molecular Weight: 408.88	377.29 (-CH_3OH), 320.0 (-$OHC_2H_4NH_2$; –C_2H_4), 288.17(-$OHC_2H_4NH_2$; –C_2H_3OH; –CH_4), 176.11 (-CH_3; $C_2H_5NH_2$; C_2H_5OH; C_6H_5Cl; –CH_3OH)	UV-$Vis_{solution}$
IMP5	0.82	407.31	 Chemical Formula: $C_{20}H_{23}ClN_2O_5$ Molecular Weight: 406.86	258.14 (-CH_3OH; C_2H_5OH; $CH_2OC_2H_4NH_2$), 230.13 (-CH_3OH; C_2H_5OH; $CH_2OC_2H_4NH_2$; –CO), 167.14 (-C_6H_5Cl; –$HCOOC_2H_4$; -$C_2H_4NH_2$; –CH_4)	Proton, Gamma, UV-Vis_{solid}, UV-$Vis_{solution}$,
IMP6	0.88	406.28	 Chemical Formula: $C_{21}H_{24}ClNO_5$ Molecular Weight: 405.87	278.19 (-C_6H_5Cl; –CH_4), 233.29 (-C_6H_5Cl; –CH_4; –C_2H_5OH), 177.20 (-C_6H_5Cl; –CH_4; –C_2H_5OH; –$HCOOCH_3$)	Proton
IMP9	1.07	408.3	 Chemical Formula: $C_{20}H_{22}ClNO_6$ Molecular Weight: 407.84	294.21 (-$HCOOCH_3$; –C_2H_6; –CH_3; H_2O) 233.29 (-$HCOOCH_3$; –$HCOOC_2H_4$; –C_2H_4OH) 177.19 (-CH_3OH; – $2C_2H_4OH$)	Gamma
IMP11	1.15	410.31	 Chemical Formula: $C_{20}H_{24}ClNO_6$ Molecular Weight: 409.86	363.25 (-C_2H_4OH), 349.18 (-$HCOOCH_3$), 244.15 (-$HCOOCH_3$; –CH_3OH; –$C_2H_5O_2$), 233.29 (-$HCOOCH_3$; –$HCOOC_2H_4$; –C_2H_4OH)	Gamma

(*continued on next page*)

Table 2 (*continued*)

Peak	RRT	Measured mass (*m/z*)	Proposed chemical structure	MS/MS fragmentation (*m/z*)	Observed in irradiated conditions
IMP12	1.18	348.25	 Chemical Formula: $C_{18}H_{21}NO_6$ Molecular Weight: 347.36	253.18 (-C_6H_5OH), 209.17 (-C_6H_5OH; –C_2H_5OH), 180.13 (-C_6H_5OH; –CH_3OH; –C_2H_5OH), 152.12 (-C_6H_5OH; –CH_3OH; –C_2H_5OH; –CO)	Gamma
IMP13	1.32	360.26	 Chemical Formula: $C_{19}H_{18}ClNO_4$ Molecular Weight: 359.80	318.28 (-CH_4; –CO), 286.18 (-$HCOOC_2H_5$), 252.20 (-$HCOOC_2H_5$; −Cl), 224.20 (-$HCOOC_2H_5$; -Cl; –CO)	Proton

$UV\text{-}Vis_{solid}$: Amlodipine besylate API irradiated by UV–Visible radiation in solid state;
$UV\text{-}Vis_{solution}$: Amlodipine besylate API irradiated by UV–Visible radiation in aqueous solution state.

in Fig. 5. The identification results suggested that the reactivity of amlodipine was due to the presence of the dihydropyridine ring and the amino group present in the molecule. The type of reactions occurred in amlodipine after photodegradation, proton and gamma irradiations included oxidation, bond rupture and hydrolysis. The oxidative aromatization of the dihydropyridine ring to the pyridine ring was one of the main degradation pathways for amlodipine. It was promoted by radiation in both solid and solution conditions. The other pathway for degradation of amlodipine was the intramolecular cyclization of the side chain in the presence of the amino group.

In the sum of all, this study provides data regarding the stability of amlodipine besylate after proton, gamma, neutrons and ^{56}Fe radiation exposure at selected doses. We have investigated that proton and gamma radiation exposure can degrade amlodipine besylate at the selected doses. Although the energy of ionizing radiation used in this study was very less compared to space radiations and doses were higher than terminal doses for humans, this study can give us an idea about the behaviour of amlodipine besylate degradation in simulated space radiation conditions i.e. accelerated or stress degradation study. It enables to understand chemical degradation behaviour of the drug following selected ionizing radiations and the toxicity potential of possible radiolytic products that can be harmful. The knowledge of the effect of ionizing radiations on the stability of the drugs can be used to prepare a more stable formulation using countermeasures described in our previous paper (Mehta and Bhayani, 2017) for future medicines for use in long-duration space missions.

4. Conclusions

The effects of ionizing radiations such as proton, neutron, gamma and ^{56}Fe were evaluated on the physicochemical stability of amlodipine besylate API and tablets by organoleptic evaluation, FTIR and HPLC studies. The results showed the dose-dependent physical and chemical changes after proton and gamma irradiations in the solid and aqueous solution state, respectively. One additional peak at 1728 cm^{-1} was observed in the FTIR spectrum of proton irradiated API due to the morpholine ring containing impurities. HPLC chromatograms revealed that amlodipine degraded up to ~10% in proton irradiated solid API and up to ~21% in gamma irradiated aqueous solution at the highest radiation doses. The drug was stable to the thermal neutron, fast neutron and ^{56}Fe radiations in the solid state conditions. Very small chemical changes (~1%) were observed in tablets compared to the API after proton irradiation. The comparison of the proton (solid state) and gamma (aqueous solution) radiation profile with photodegradation indicated a possibility of different pathways for the formation of radiolytic products. Total ten major degradation products were identified by LC-MS/MS and the probable drug degradation mechanism was proposed. To best of our knowledge, the structures of two radiolytic products i.e. IMP9 and IMP11 are not reported yet.

Although mild degradation was observed after ionizing radiation exposure, it was important to document to provide evidence that the ionizing radiation exposure leads degradation of medicines. However, more extensive and systematic studies are required as space radiation is even harsher in terms of radiation energy, which may pose more significant damaging effects on medicines during a future long-duration space mission. The knowledge about the degradation of the drug upon radiation exposure allows taking crucial steps to enhance the drug stability. Moreover, it permits to select a stable molecule for pharmacotherapy. Different protective packaging or optimization of formulation development can help in the prevention of degradation of the drug in the presence of radiation.

CRediT authorship contribution statement

Dhara Bhayani: Methodology, Investigation, Data curation, Writing - original draft, Visualization. **Haladhara Naik:**Resources, Methodology. **T. Newton Nathaniel:**Resources, Methodology. **Saif Khan:**Resources, Methodology. **Priti Mehta:**Conceptualization, Methodology, Visualization, Supervision.

Declaration of Competing Interest

There are no conflicts to declare. This article does not contain any studies with human participants or animals performed by any of the authors.

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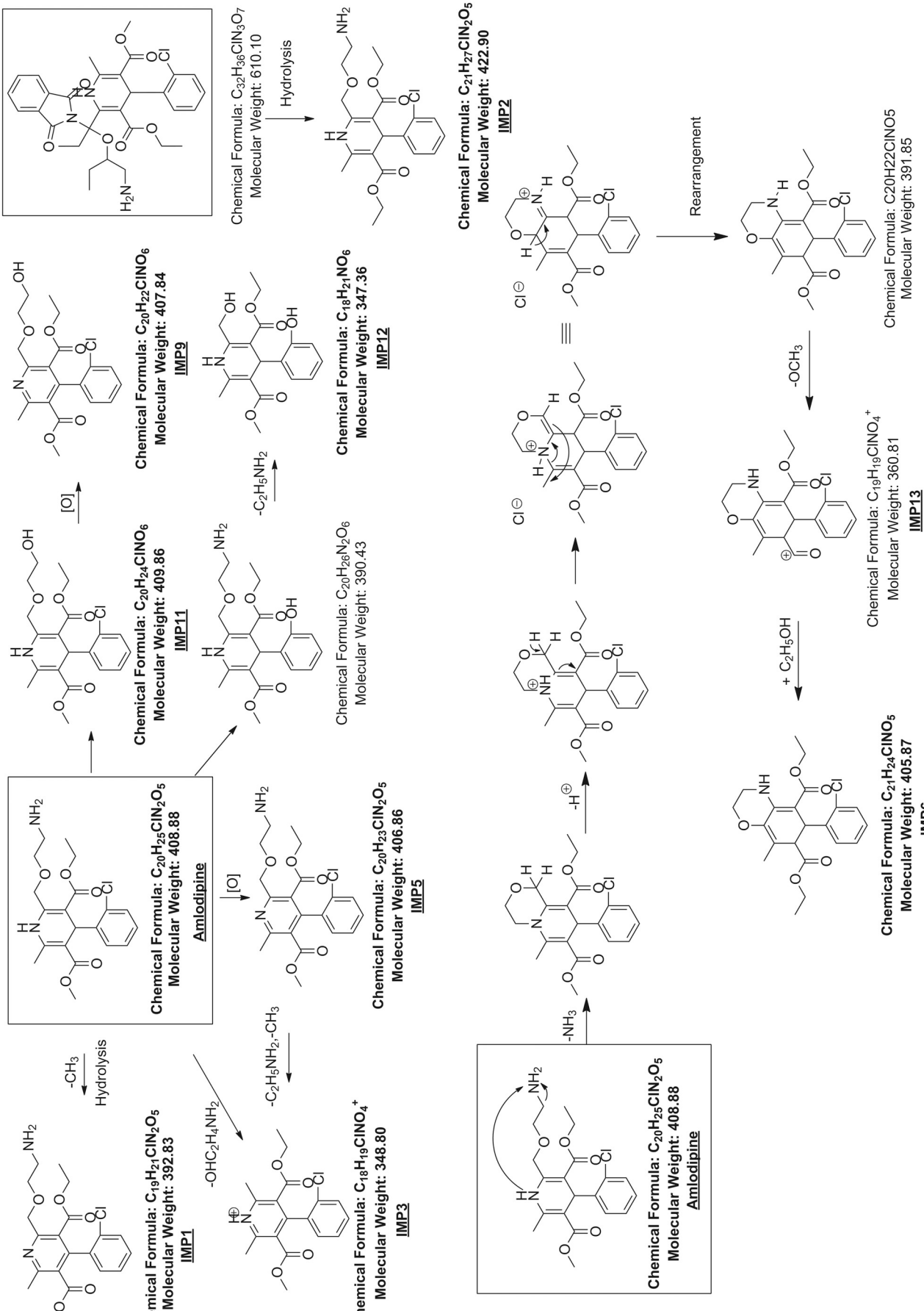

Fig. 5. Radiation degradation scheme of amlodipine besylate.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejps.2019.104982.

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